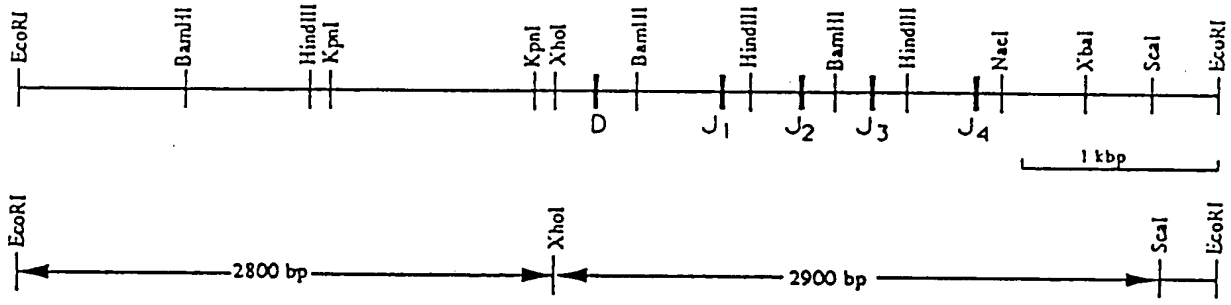
Mouse Heavy Chain J Genes Inactivation Vector

(A) Targeted mouse heavy chain J genes



(B) Inactivation vector mDΔJ.Neo

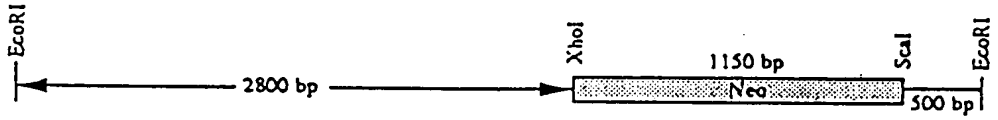
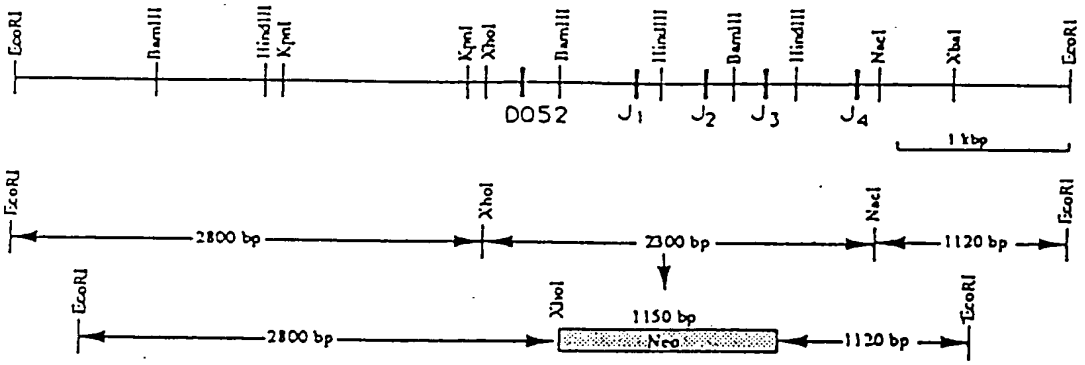
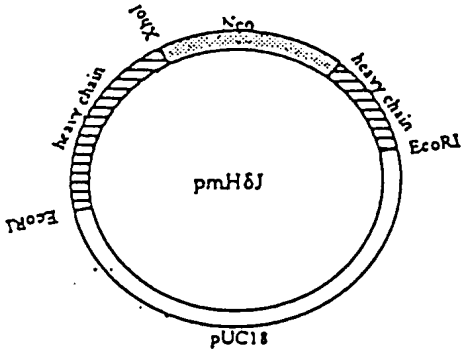


Figure 1

(A) Targeted mouse heavy chain J genes

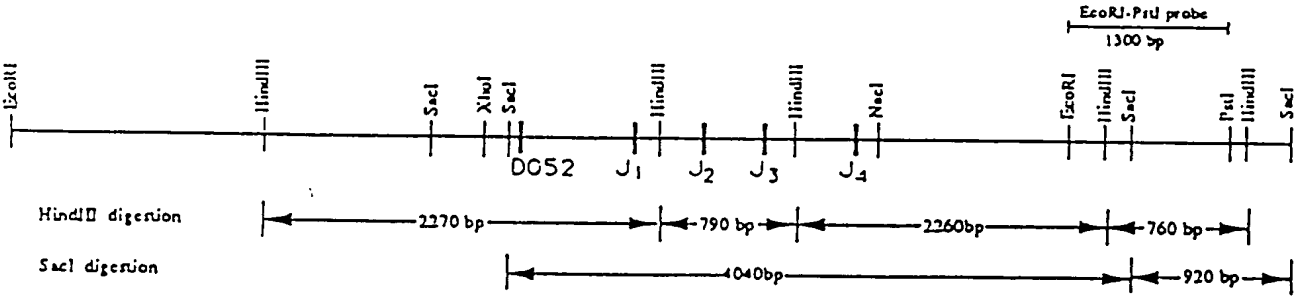


(B) Inactivation vector pmHδJ



(C) Southern analysis of pmHδJ-targeted ES colonies

Wild type ES cell genome



Targeted ES cell genome

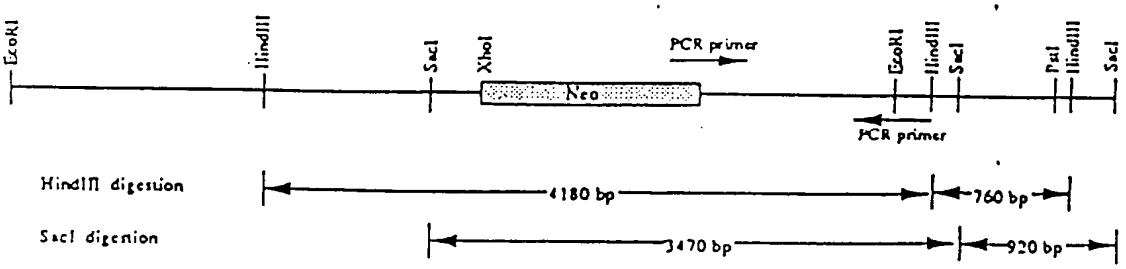


Figure 2

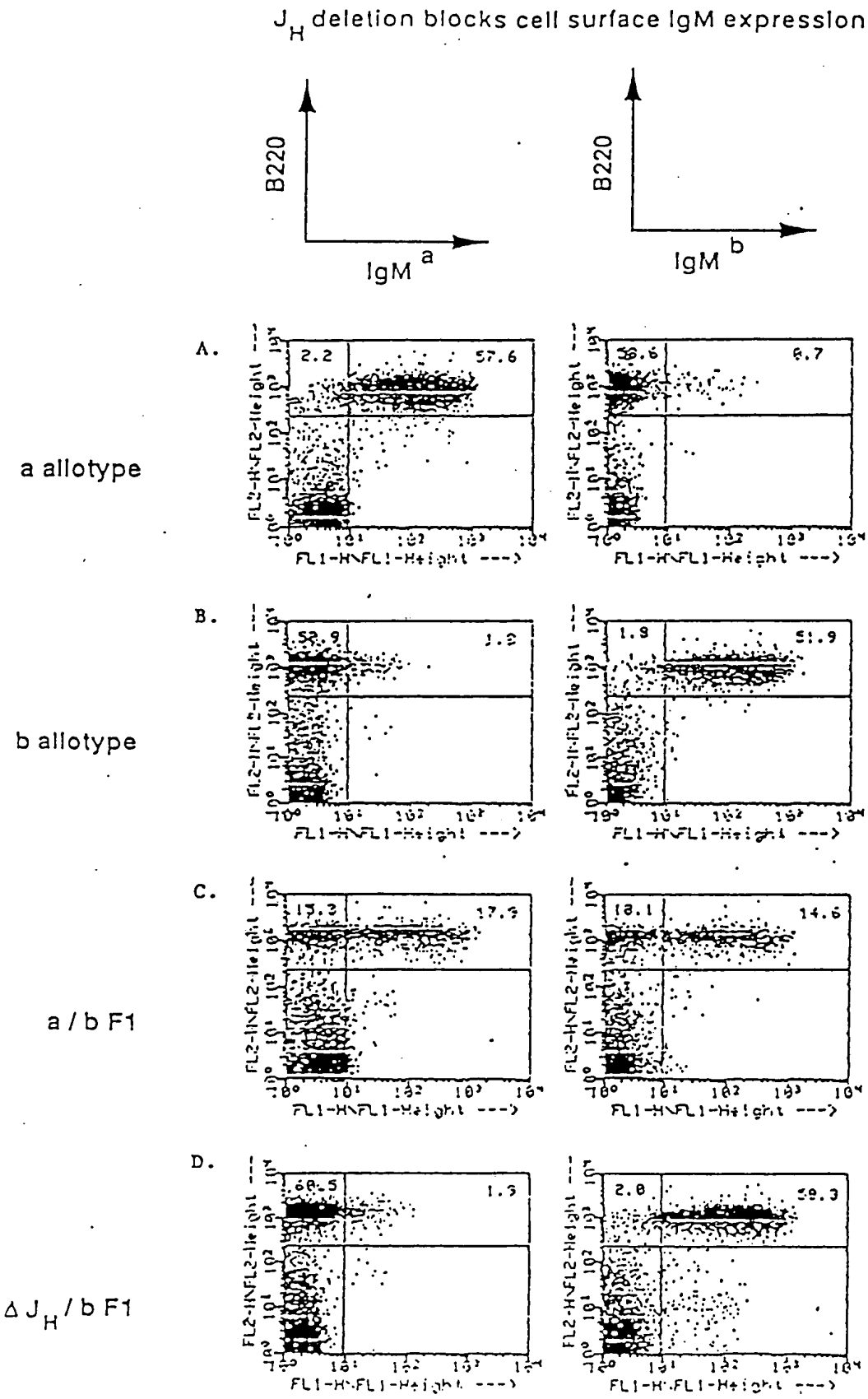

J H deletion blocks cell surface IgM expression
B220
IgM a
B220
IgM b
A.
a allotype
2.2
57.6
6.6
6.7
B.
b allotype
52.9
1.8
1.9
51.9
C.
a / b F1
15.3
17.3
18.1
14.6
D.
Δ J H / b F1
68.5
1.6
2.8
29.3
FL2-H:FL2-Height --->
FL1-H:FL1-Height --->

Figure 3

Staining of peripheral blood lymphocytes with fluorescent anti-a allotype (A, D), anti-b allotype (B,E) or anti-B220 (C, F). (A, B, C) JH-deletion homozygous mutant mouse 244-3-2/F2-7, (D) A allotype control mouse, (E) B allotype control (F) control mouse. The number in each panel indicates the percentage of cells stained with the specific antibody.

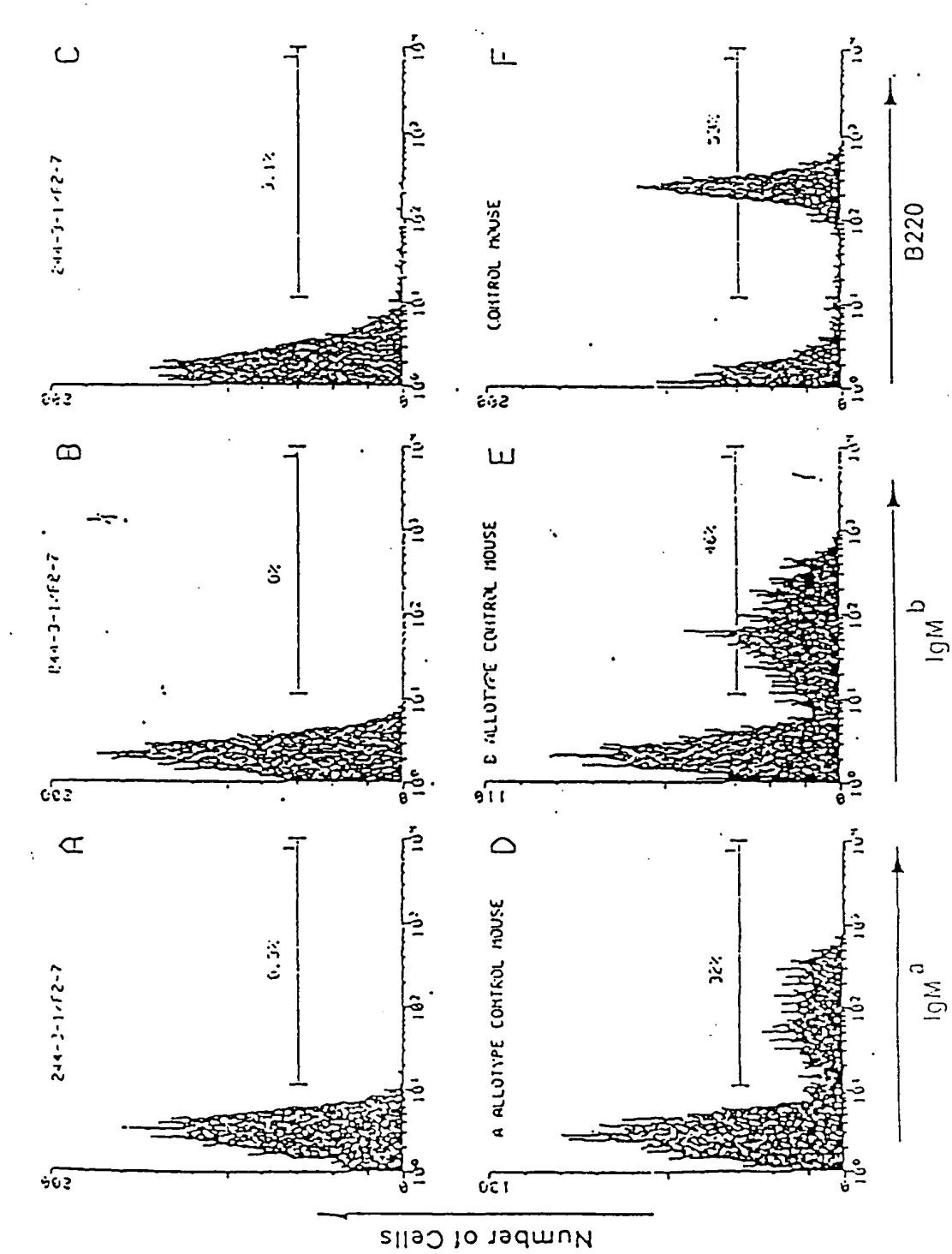


Figure 4

INACTIVATION OF KAPPA CONSTANT REGION

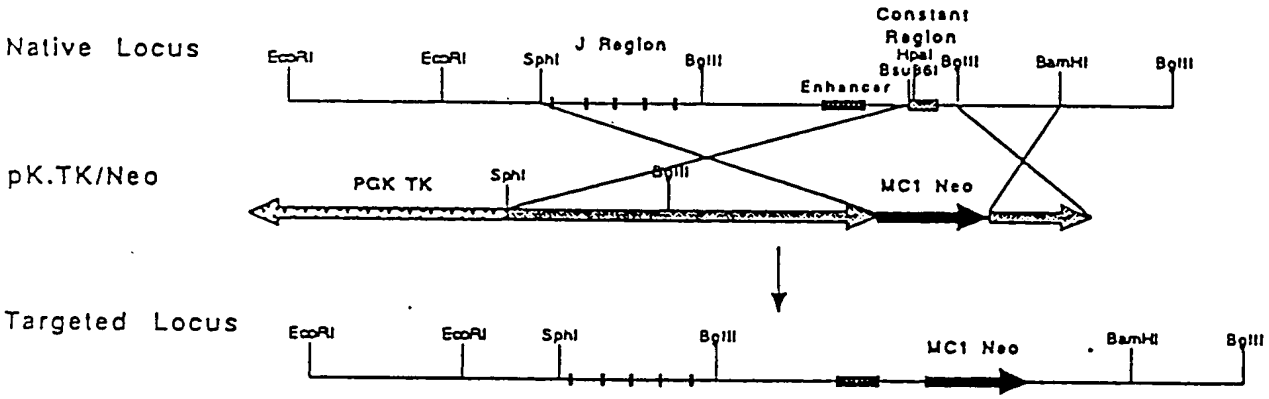


Figure 5

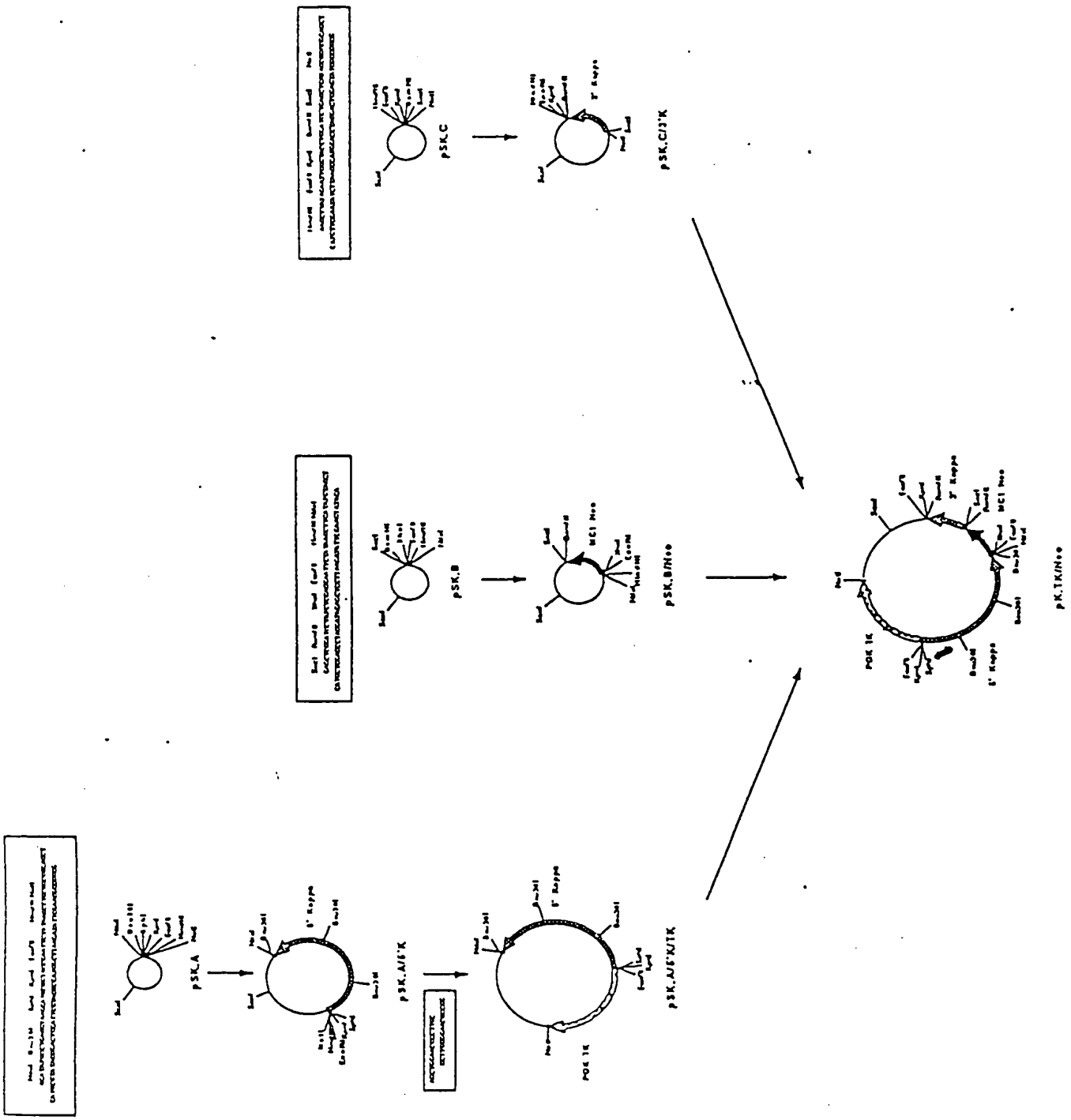


Figure 6

SOUTHERN ANALYSIS OF LIGHT CHAIN Cκ-TARGETED E14-1 CELLS

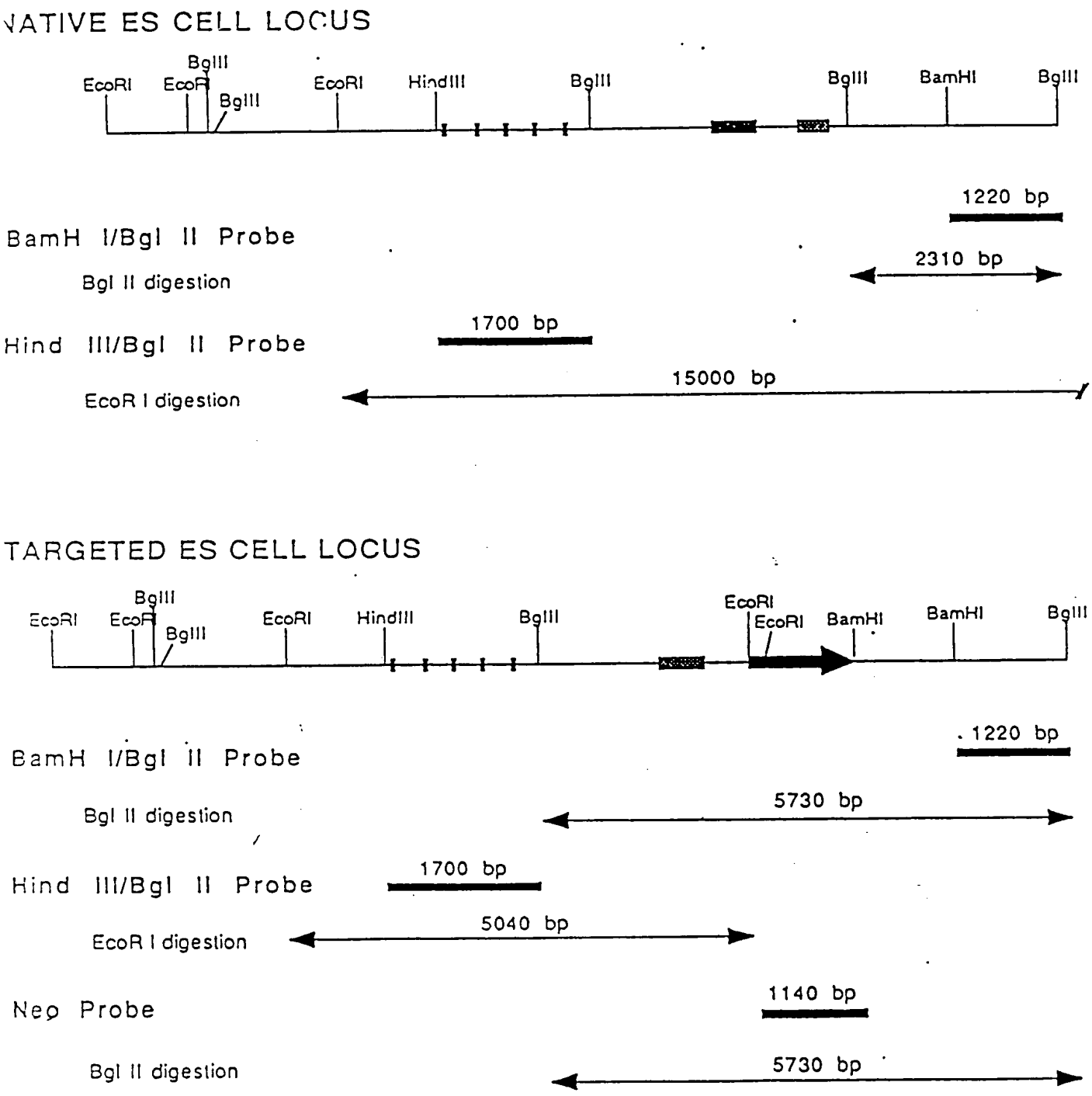
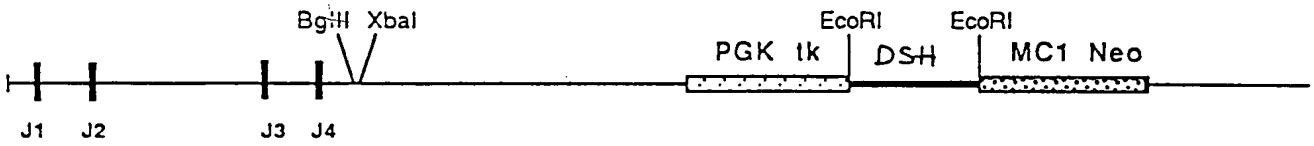


Figure 7

KAPPA J/CONSTANT REGION INACTIVATION

J REGION KNOCKOUT VECTOR



TARGETING SCHEME

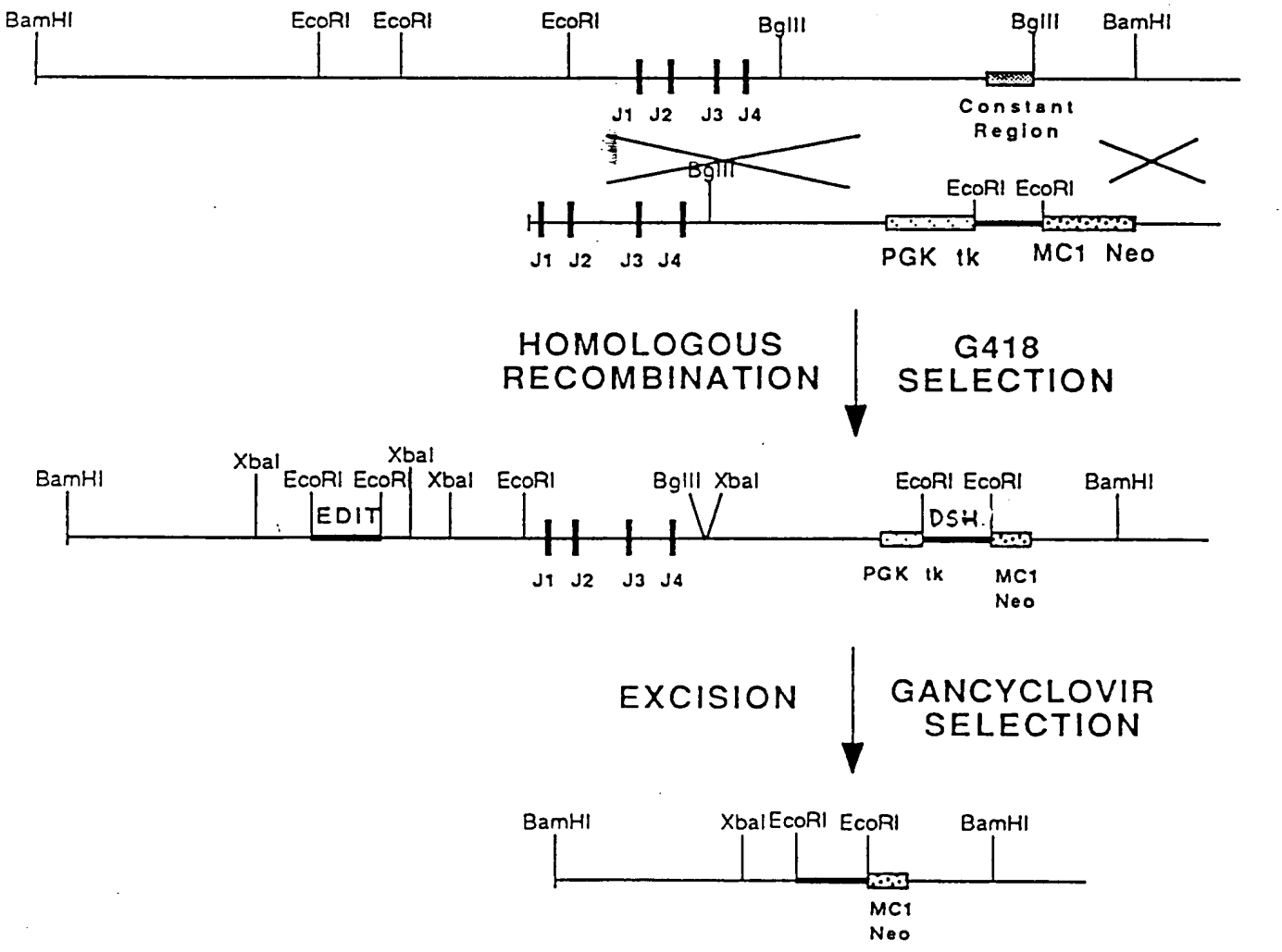


Figure 8

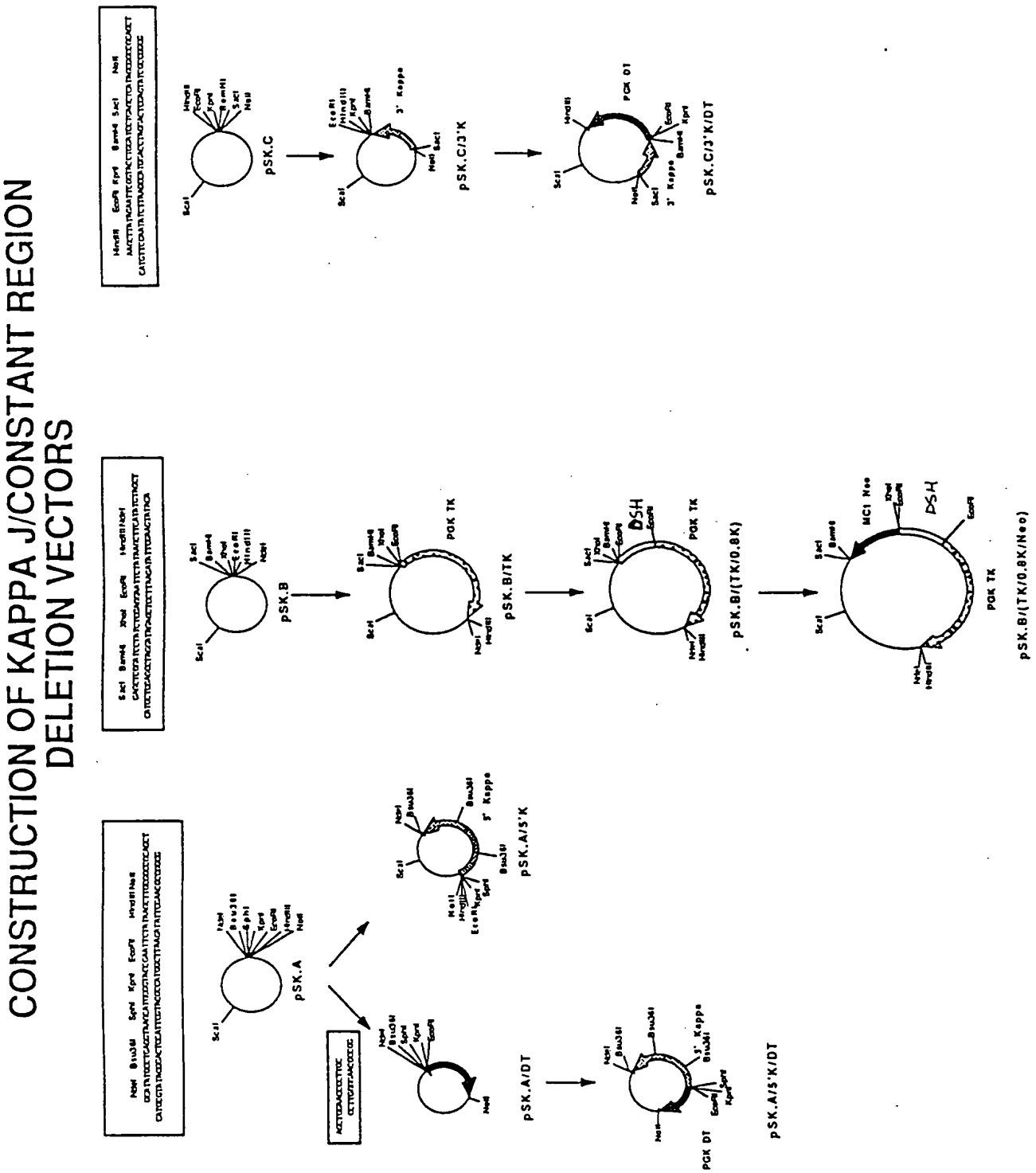
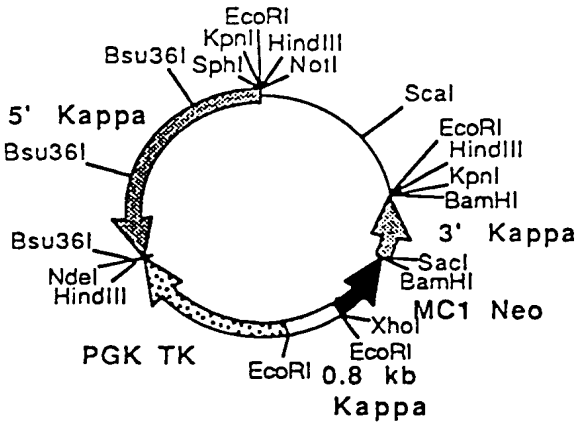
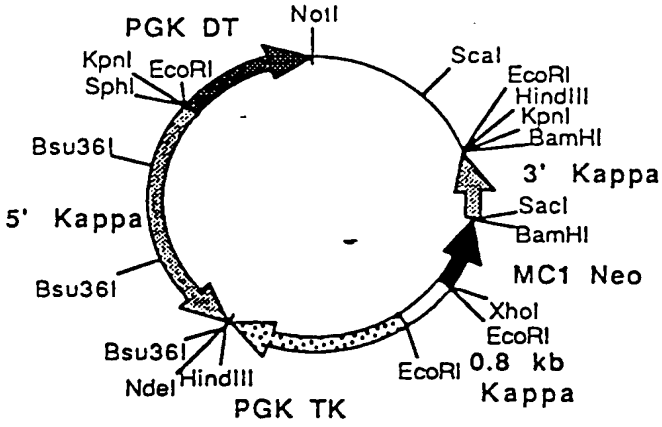

CONSTRUCTION OF KAPPA J/CONSTANT REGION DELETION VECTORS
pSK.C
pSK.C/J'K
pSK.C/J'K/DT
PGK DT
3' Kappa
pSK.B
pSK.B/TK
pSK.B/(TK/0.8K)
pSK.B/(TK/0.8K/Neo)
PGK TK
DSH
MC1 Neo
pSK.A
pSK.A/5'K
pSK.A/DT
pSK.A/5'K/DT

Figure 9

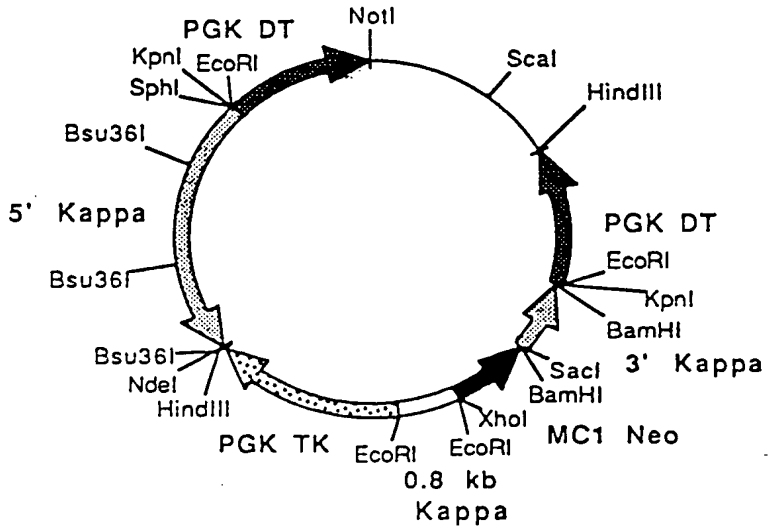
KAPPA J/CONSTANT REGION DELETION VECTORS



pK.(TK/0.8K/Neo)



pK.DT/(TK/0.8K/Neo)



pK.DT/(TK/0.8K/Neo)/DT

Figure 10

SOUTHERN ANALYSIS OF LIGHT CHAIN Jκ/Cκ-DELETED E14-1 CELLS

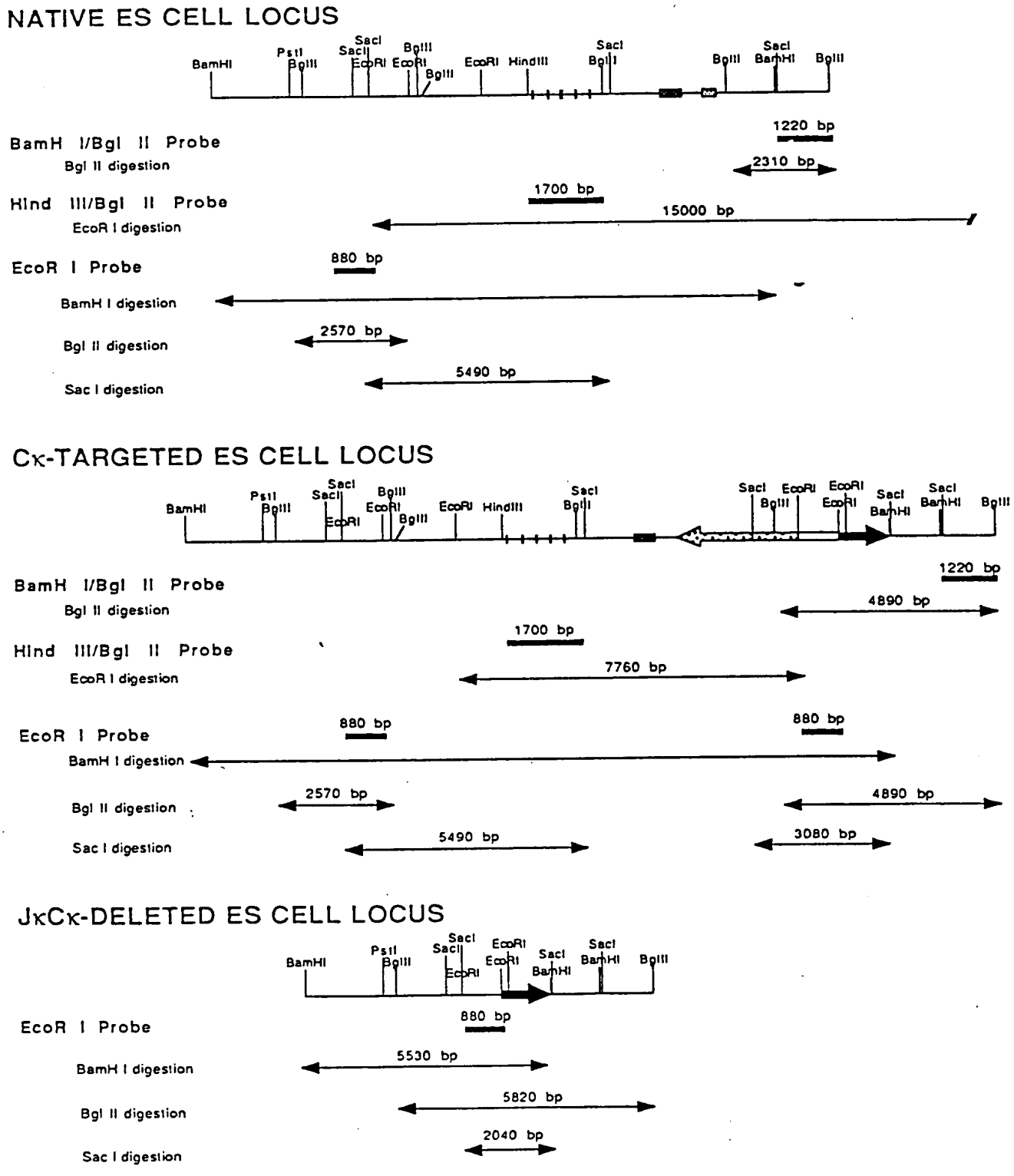


Figure 11

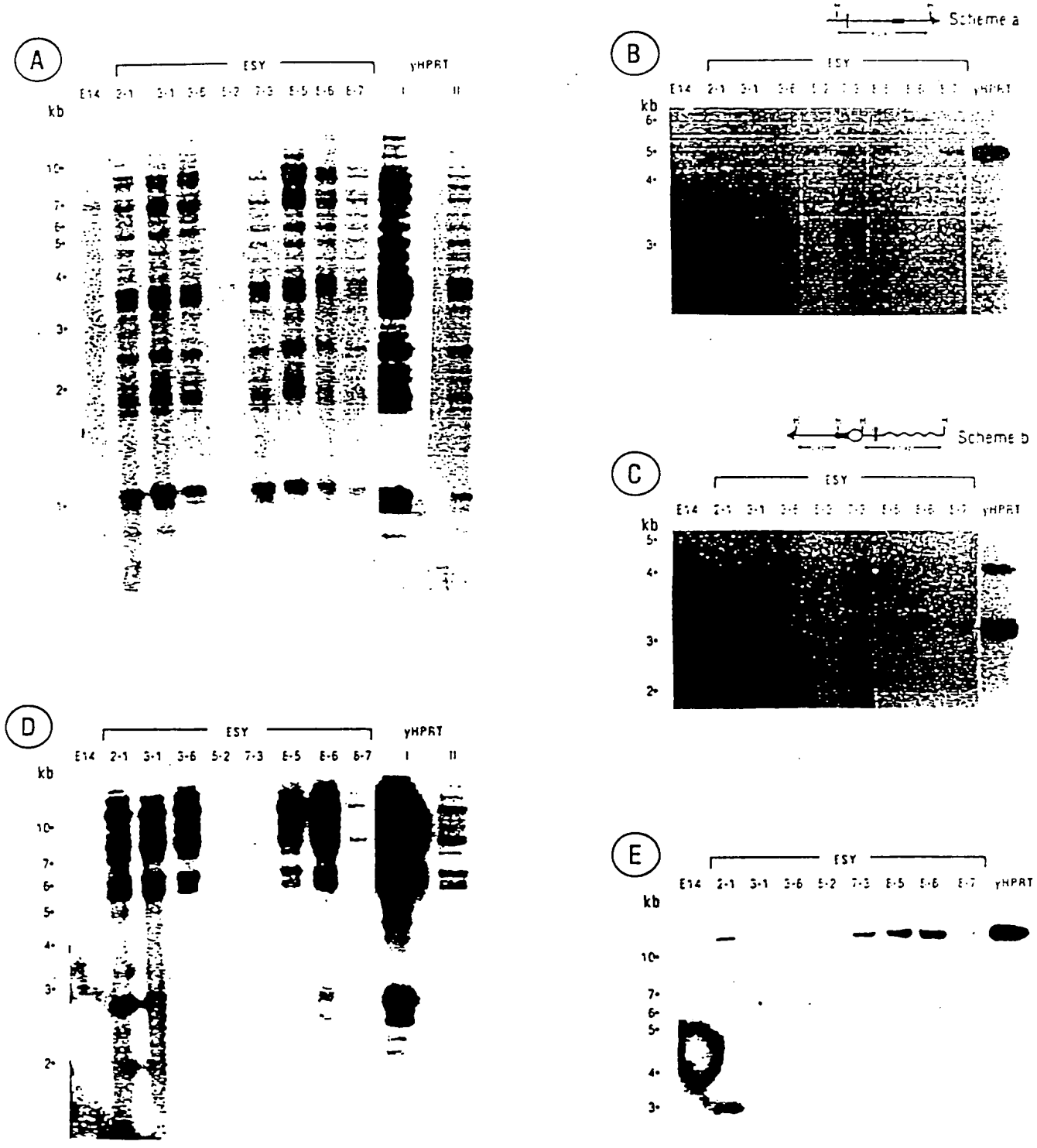

A
ESY
yHPRT
E14 2-1 3-1 3-6 5-2 7-3 8-5 8-6 8-7 I II
kb
10
7
6
5
4
3
2
1
B
Scheme a
ESY
E14 2-1 3-1 3-6 5-2 7-3 8-5 8-6 8-7 yHPRT
kb
6
5
4
3
C
Scheme b
ESY
E14 2-1 3-1 3-6 5-2 7-3 8-5 8-6 8-7 yHPRT
kb
5
4
3
2
D
ESY
yHPRT
E14 2-1 3-1 3-6 5-2 7-3 8-5 8-6 8-7 I II
kb
10
7
6
5
4
3
2
E
ESY
E14 2-1 3-1 3-6 5-2 7-3 8-5 8-6 8-7 yHPRT
kb
10
7
6
5
4
3

Figure 12

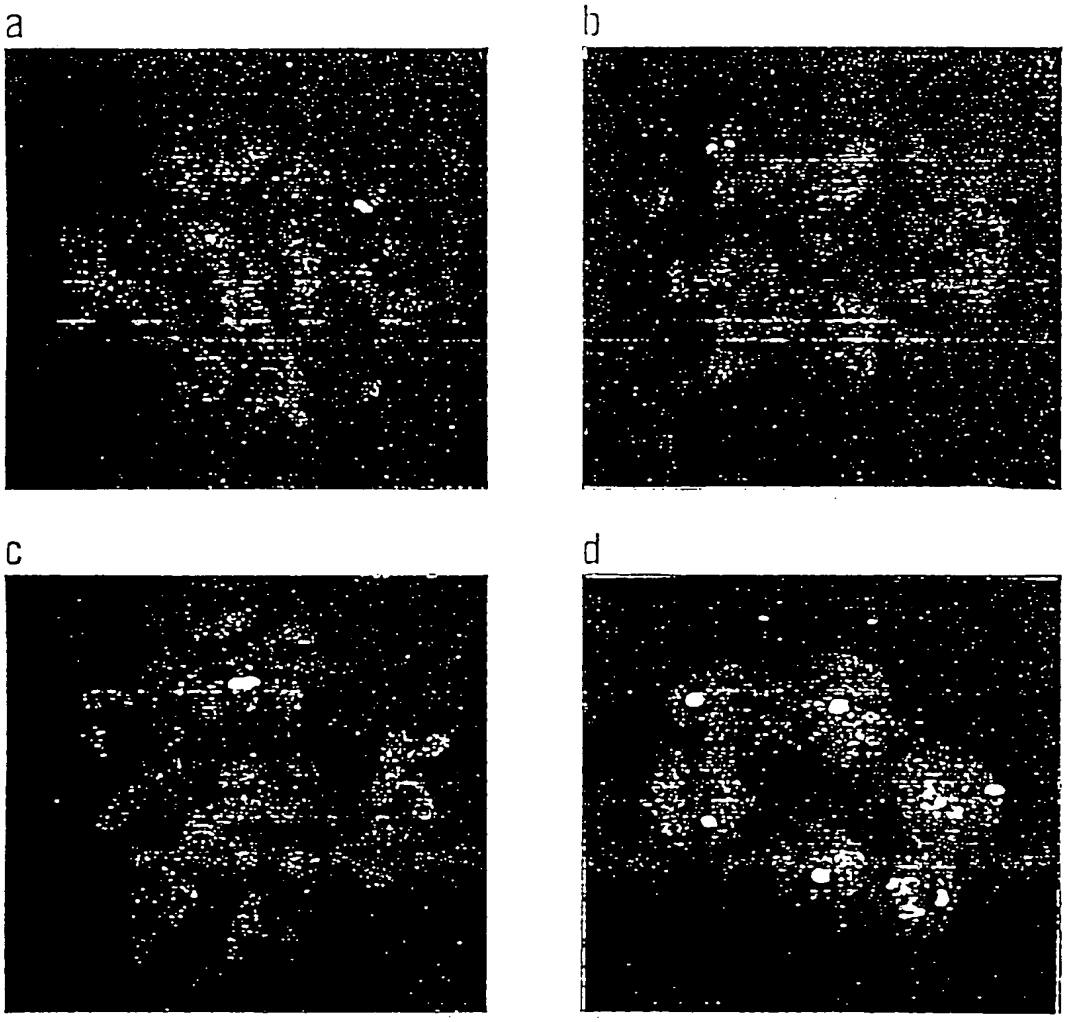
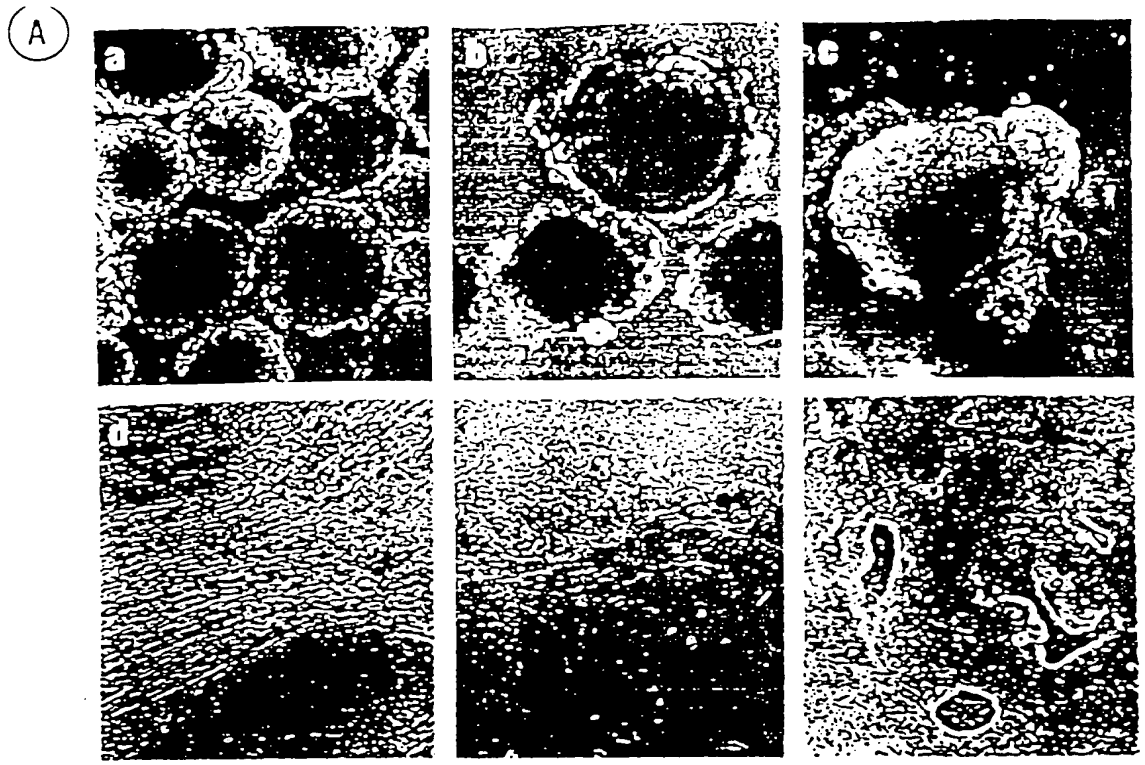
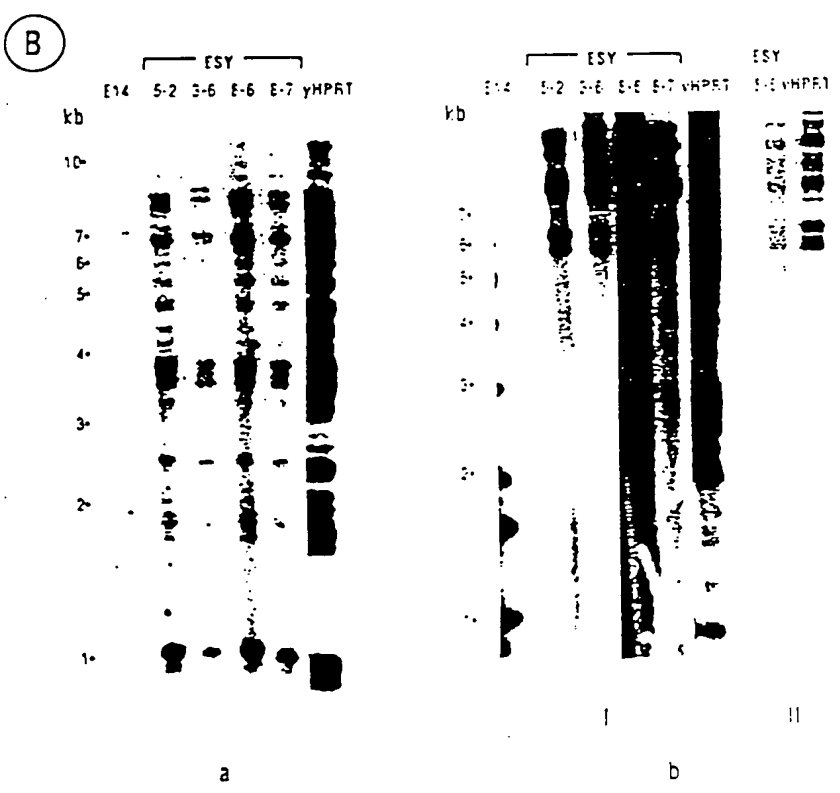


Figure 13


A
a
b
c
d


B
ESY
E14
5-2
3-6
E-6
E-7
yHPRT
kb
10
7
5
4
3
2
1
a
b
I
II

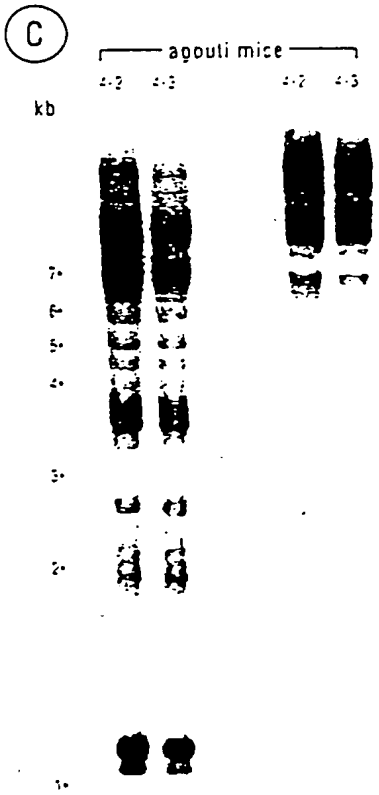

C
agouti mice
kb
7
5
4
3
2
1

Figure 14

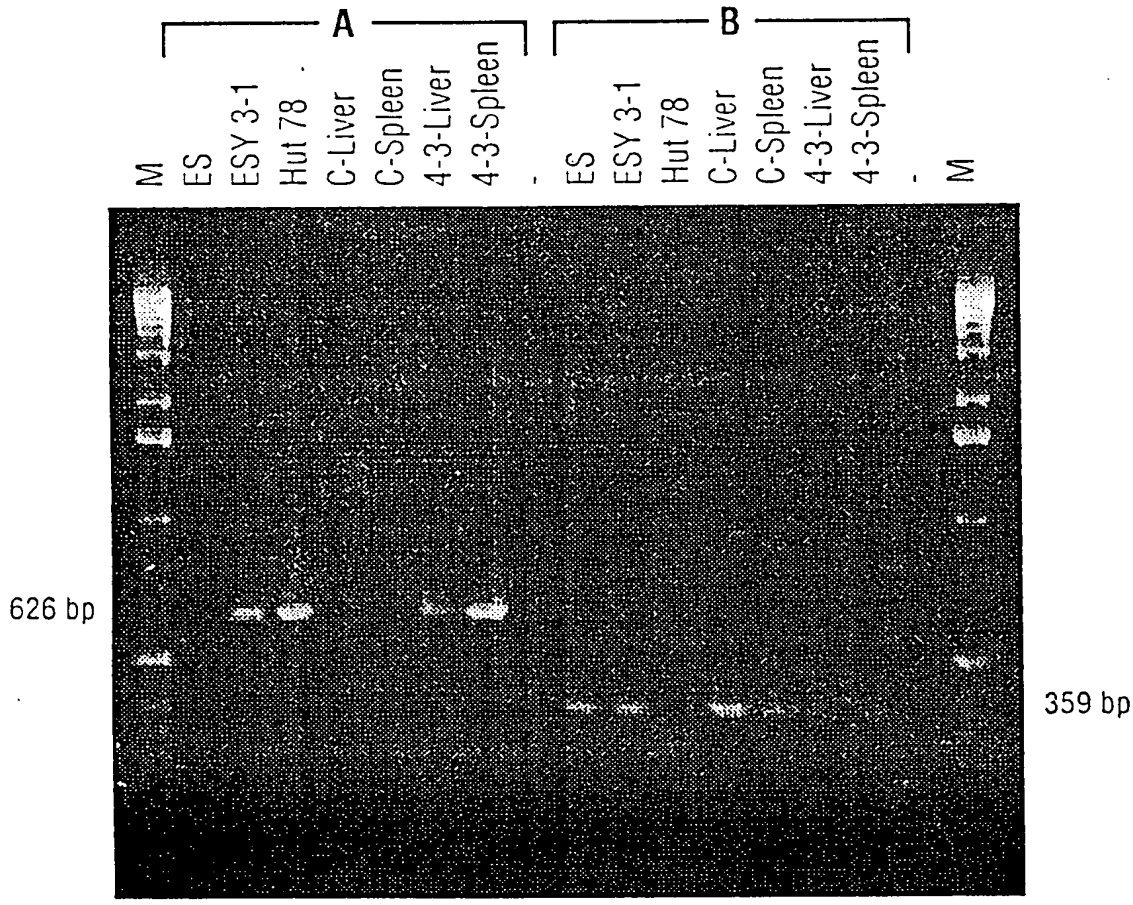
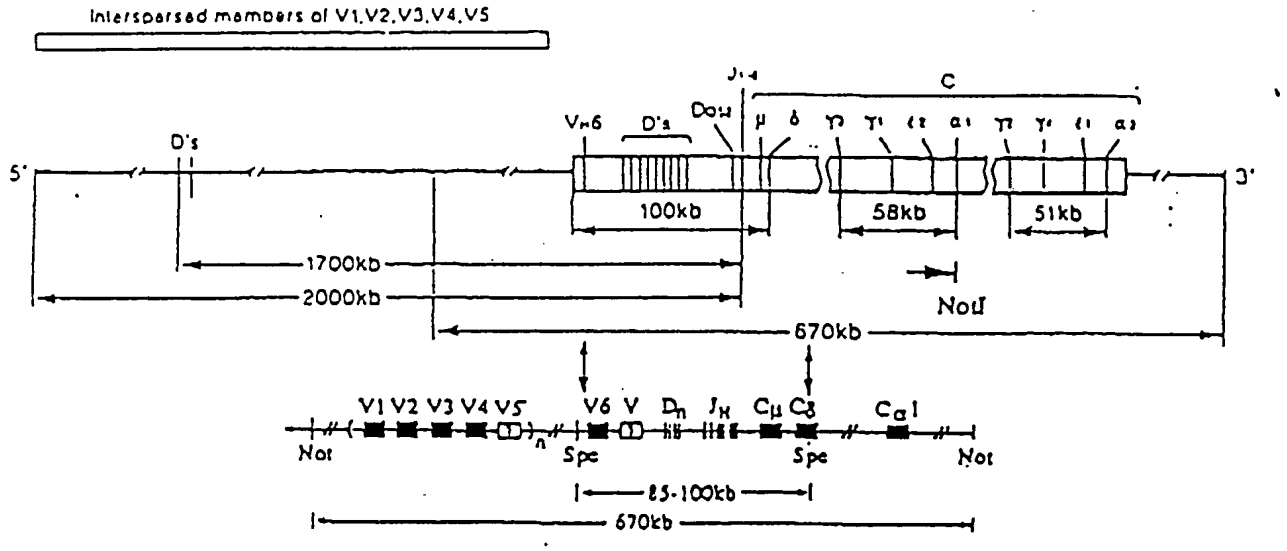
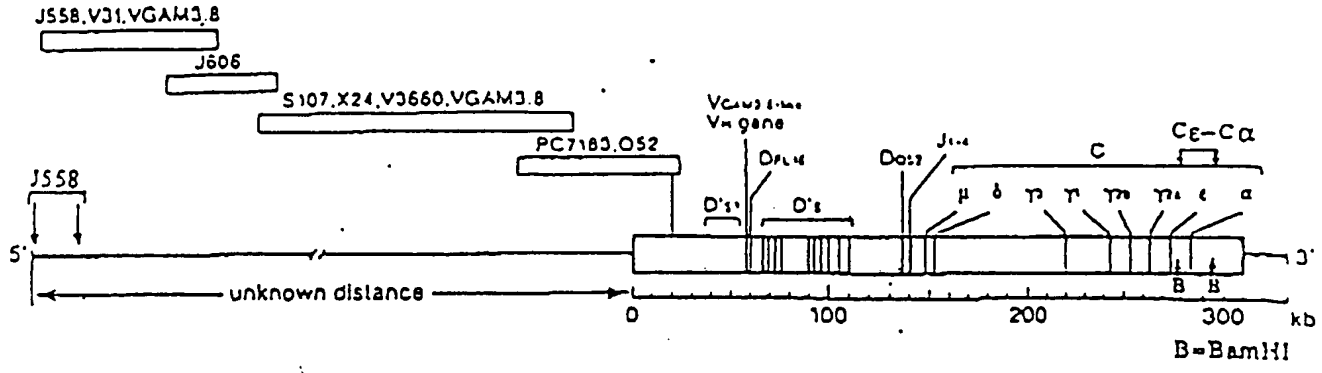

A
B
M
ES
ESY 3-1
Hut 78
C-Liver
C-Spleen
4-3-Liver
4-3-Spleen
-
ES
ESY 3-1
Hut 78
C-Liver
C-Spleen
4-3-Liver
4-3-Spleen
-
M
626 bp
359 bp

Figure 15

(A) Human heavy chain locus



(B) Mouse heavy chain locus



(C) Human heavy chain replacement YAC vector

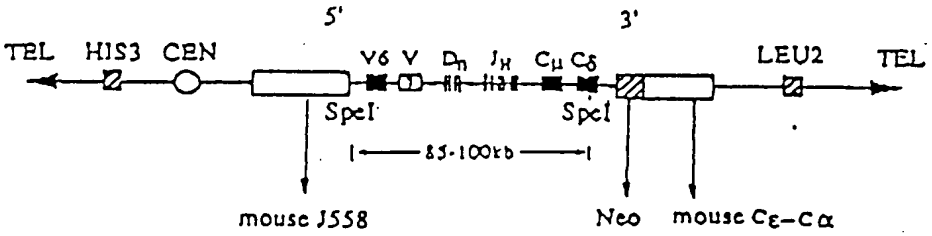


Figure 16

Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X
heterozygous inactive Murine IgK

MIgH (inactive) MIgK
MIgH MIgK
X
MIgH MIgK (inactive)
MIgH MIgK

↓

F1 (cross I A)

MIgH (inactive) MIgK (inactive)
MIgH MIgK

Cross I B.

heterozygous Human IgH
X
heterozygous Human IgK

MIgH MIgK HIgH
MIgH MIgK
X
MIgH MIgK HIgK
MIgH MIgK

↓

F1 (cross I B)

MIgH MIgK HIgH HIgK
MIgH MIgK

Cross II. F1 (cross I A) x F1 (cross I B)

↓

F2 Quadruple Heterozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH MIgK

Cross III. Intercross F2 mice

↓

F3 DOUBLE Homozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH (inactive) MIgK (inactive)

Figure 17

MAMMALIAN HOST GENOTYPES

	Hetero- or Hemi-zygous Mice	Intercross Product Mice*
I.	ΔmIgL mIgH mIgL mIgH	ΔmIgL mIgH ΔmIgL mIgH
II.	mIgL ΔmIgH mIgL mIgH	mIgL ΔmIgH mIgL ΔmIgH
III.	mIgL mIgH hIgH mIgL mIgH	mIgL mIgH hIgH mIgL mIgH hIgH
IV.	mIgL mIgH hIgL mIgL mIgH	mIgL mIgH hIgL mIgL mIgH hIgL
V.	Animal I X Animal II	
	ΔmIgL mIgH mIgL ΔmIgH	ΔmIgL ΔmIgH ΔmIgL ΔmIgH
VI.	Animal III X Animal V	
	mIgL mIgH hIgH ΔmIgL ΔmIgh	ΔmIgL ΔmIgH hIgH and ΔmIgL ΔmIgH hIgH ΔmIgL ΔmIgH hIgH ΔmIgL ΔmIgH
VII.	Animal IV X Animal V	
	mIgL mIgH hIgL ΔmIgL ΔmIgH	ΔmIgL ΔmIgH hIgL and ΔmIgL ΔmIgH hIgL ΔmIgL ΔmIgH hIgL ΔmIgL ΔmIgH
VIII.	Animal VI X Anmial VII	
	ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH	ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH hIgL hIgH
	mIgL mIgH hIgL hIgH ΔmIgL ΔmIgH	ΔmIgL ΔmIgH hIgL hIgH and ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH
IX.	Animal III X Animal IV	
	mIgL mIgH hIgL hIgH mIgL mIgH	mIgL mIgH hIgL hIgH mIgL mIgH hIgL hIgH
X.	Animal II X Animal IX	
	mIgL ΔmIgH hIgL hIgH mIgL mIgH	mIgL ΔmIgH hIgL hIgH and mIgL ΔmIgH hIgL hIgH mIgL ΔmIgH hIgL hIgH mIgL ΔmIgH
XI.	Animal I X Animal IX	
	ΔmIgL mIgH hIgL hIgH mIgL mIgH	ΔmIgL mIgH hIgL hIgH and ΔmIgL mIgH hIgL hIgH ΔmIgL mIgH hIgL hIgH ΔmIgL mIgH

*Not all possible genotypes from intercrosses are shown.

Δ = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain

FIGURE 18